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Biomineralisation of Organophosphorus Pesticide (Malathion) using Fungal Species *Rhizopus oligosporus* from Cotton Soils of Andhra Pradesh, India

P. Udaya Sri[1]*, K.R.S. Sambasiva Rao[1] and K.M. Subbu Rathinam[2]

[1]Department of Biotechnology, Acharya Nagarjuna University, Nagarjuna Nagar - 522 510, India.
[2]Department of Zoology, Rajah Serfoji Government College, Thanjavur - 613 005, India.

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Cotton is the world's most important non-food agricultural commodity, which is easily damaged by various sucking pests like jassids, aphids, thrips, whitefly, red spider, mites, mealy bug and bollworms. Pesticides and their residues pose a major problem by their accumulation in different parts of the world in general and in different biological organisms of the food chain in particular. In the light of the importance and essentiality to overcome the deleterious effects of pesticides, the present study has made an attempt to isolate the fungal species capable of degrading organophosphorus pesticides (chlorpyriphos, malathion and triazophos) that are widely sprayed on cotton crop in Andhra Pradesh and to develop a model for the control of pesticide pollution. The isolated species were identified basing on microscopic structural and growth characterization studies together with scanning electron microscopy and the molecular characterization was also done basing on the sequence analysis of the DNA coding for 18s rRNA of the test fungal organism. The results may fit the findings of others on the ability of some fungi to degrade pesticides and introduce some new information on mineralization of the organic constituents of pesticides. It was elucidated that the selected fungal species is an effective biodegrading agent and by optimizing the different cultural conditions and by using large scale culture techniques, this fungal species, *Rhizopus oligosporus,* can be very effectively used as biodegrading agent in cotton fields as effective biocleaner and will be an economically effective microbial process which is an alternative for control of environmental pollution.

Key words: Pesticides, Organophosphates, Biodegradation, Environmental pollution.

India is primarily an agriculture-based country with more than 60-70% of its population dependent on agriculture. A tremendous pressure will be exerted on the annual food grain production and on minimizing the crop losses. About 30% of agriculture produce is lost due to pests. Hence agrochemicals like insecticides, fungicides, pesticides and herbicides have increasing demand for protection of crops in one way or the other and large numbers of pesticides are emerging out day by day.

Cotton is the world's most important non-food agricultural commodity, but in India it is one of the important commercial fiber crops of South India. It is the back bone of textile industry accounting for 70% of total fiber consumption in the textile sector and 38.5% of the country's export, fetching income of Rs. 42,000 crores. Cotton is easily damaged by various sucking pests like jassids, aphids, thrips, whitefly, redspider, mites, mealy bug and Bollworms (Pimentel, 1983). This accounts for the consumption of more amounts of

* To whom all correspondence should be addressed.
E-mail: pudayasri@gmail.com

insecticides on cotton than any other single crop that is the reason why cotton growers are always looking for ways to protect their crops. There is a tremendous need to increase the agricultural production to meet the requirement of increasing population. Pesticides have gained an immense importance for the very effective control of agricultural pests which spoils 1/3rd of food production. The indiscriminate use of pesticides for pest control has resulted in unprecedented chemical pollution and simultaneously affecting the non-target organisms (Kurzel and Cetrulo, 1981; Hayes, 1986). Pesticides and their residues pose a major problem by their accumulation in different parts of the globe in general and in different biological organisms of the food chain in particular (Singh *et al.,* 1987).

In the light of the importance and essentiality to overcome the deleterious effects of pesticides, an attempt has been made in the present study to investigate the possibility of biodegradation of organophosphorus pesticides using fungal species and to develop a model for control of pesticide pollution.

MATERIAL AND METHODS

Distribution of cotton cultivable area in Guntur District of Andhra Pradesh

A Survey of cotton cultivable area was carried out in Guntur District of Andhra Pradesh. Soil samples were collected from different areas by adopting simple random sampling technique to isolate the pesticide degrading fungi from the soils where tremendous pesticide consumption had taken place. Soil samples were collected from Siripuram, Prathipadu, Chilakaluripet and Amaravathi of Guntur District, which is the leading district in the cultivation of cotton in Andhra Pradesh. Information was obtained from farmers regarding the extensively sprayed pesticides on cotton crop.

Isolation and identification of fungal strains

The soil samples were collected at a depth of 15 inches depth from the soil where there was high moisture content, because the fungi need moisture content for growth. The soil sample collected was dispensed in the sterile bags and sealed and the samples were brought to the laboratory and were added with 1% of the selected insecticide in addition to controls. After incubation for 4 weeks at 28ºC, soil samples for isolation of fungi were taken using the dilution plate method. The isolated fungi were identified using Lactophenol cotton blue staining technique, scanning electron microscopy and molecular techniques.

Lactophenol cotton blue Staining

The fresh culture of different age starting from 5 to 25 days was used for the preparation of semi-permanent slides. A small bit of mycelium was placed on the slide and mixed with a drop of Lacto phenol cotton blue. Then it was slightly heated on the steam and fixed. Finally the stained slide was covered with cover slip and observed under binocular compound microscope.

Scanning electron microscopy of test fungi

Scanning Electron microscopic studies was carried out by taking 24 h old cultures of test fungi and fixed in 6% buffered Glutaraldehyde followed by post fixation in Osmium tetroxide and then dehydrated in increasing concentration of Ethyl alcohol. The samples were mounted on copper stubs with double sided adhesive tape, coated with gold polaron, AU/PD sputter-coater and scanned in SEM (Jeol JSM 5600, Japan) and photographed. The SEM studies were conducted at Ruska laboratories, Sri Venkateswara Veterinary University, Rajendranagar, Hyderabad.

Identification of the isolated fungi by sequencing of the amplified 18S rRNA gene

The most powerful tool to identify the unknown fungal species is to sequence the gene (DNA) coding for 18S rRNA, which is present in the genome of the fungi. The gene coding for the 18S rRNA is amplified using the Polymerase Chain Reaction (Mullis, 1990), and the amplified product has been subjected to sequencing and the sequence obtained has been compared with the sequence obtained from the Nucleotide Database of NCBI.

Studies on the Growth of Selected Test Fungi Determination of Growth

Growth pattern of the strain was studied in Potato Dextrose broth, Czapekdox broth & Sabourauds broth. The isolated fungal strain was inoculated into the broth and the culture was incubated at 20^0C. At every 24hrs interval, growth was noticed. The growth of the culture in broth is noticed by taking the spore counts and dry weights

at successive time intervals using the following formula:

$$\text{Formula} = \frac{\text{Total no. of spores in 5 squares}}{5} \times 10^4 \text{ spores/cm}$$

$$= \frac{I+II+III+IV+V}{5} \times 10^4 \text{ spores/cm}$$

Effect of Physico-Chemical Parameters (Temperature, pH and Salt Concentration) On the Growth of Organophosphate Degrading Fungi

The isolated fungal strain was inoculated into the Potato Dextrose broth and the culture was incubated at 20^{0}C. To assess the growth at varied pH, the pH was adjusted as 4, 5,6,7,8 and 9. To assess the growth at varied salt concentrations, the salt concentrations were adjusted as 0%, 0.5%, 5%, 7.5% and 10%. Then the flasks were autoclaved at 15 lbs pressure for 20 minutes at 121^{0}C. To assess the growth at varied temperatures, the temperatures were adjusted as 25^{0}C, 28^{0}C, 30^{0}C, 35^{0}C, 37^{0}C, 40^0 C, 45^{0}C, 55^{0}C and incubated for 4 days. (Aneja, 2003).

Confirmation of Pesticide Biodegrading ability of Fungi *Rhizopus oligosporus*

Malathion, the most widely used Organophosphate pesticide in Guntur district, particular for cotton cultivation to control cotton leaf worm was selected for the degradation studies. The isolated fungal strain was inoculated into the Potato Dextrose broth and CzapekDox medium and the culture was incubated at 20^{0}C. At every 24hrs interval, growth was noticed. The growth of the culture in broth is noticed by taking the spore counts and dry weights at successive time intervals**.** The amount of the pesticide residue in the samples was analyzed through GC technique. Residual pesticide and pesticide breakdown products extracted from the microbial degradation studies were quantified by GLC methods, using Varian- model CP 3800 with a tritium source electron capture detector and a Tracor model MT-220 with a Melpar flame photometric detector (pulsated flame photometric detector) (Ramesh Babu *et al.*, 2001).

RESULTS AND DISCUSSION

Although it is well established that the microorganisms are capable of degrading pesticides and present in all types of environment(s), still environmental pollution is a persistent problem and many areas are highly contaminated with pesticides and other chemicals (Alexander, 1994). Their susceptibility to biodegradation change drastically, depending on several factors related to the chemical and physical properties of both the pesticides and the environment in which they are present. Based on these facts, our investigation was aimed at studying the biodegradation of organophosphorus pesticides using the selected fungal species *Rhizopus oligosporus* isolated from the cotton soils of Andhra Pradesh, India.

A survey was conducted during the year 2005-2006 in various cotton cultivable areas of Guntur District of Andhra Pradesh for determining the intensity of pesticide utilization through direct interaction with the cotton farmers (Table 1).

Different soil samples were collected from different locations of cotton cultivated areas of Guntur District. As the soil contain different useful bacterial and fungal species, in our present investigation, the soil samples from different areas were screened to identify the various bacterial and fungal species present in the samples (Table 2). These soil samples consist of various fungal species of the genus *Aspergillus*, *Fusarium*, *Penicillium*, *Rhizopus*, *Trichoderma* etc., The fungal isolates were cultured in the presence of the selected widely used organophosphorus pesticides.

Microscopic structural and growth characterization, scanning electron microscopic studies and molecular characterization basing on DNA coding for 18s rRNA sequences of the test organisms capable to degrade pesticides were performed and it was concluded that the test fungus is *Rhizopus oligosporus*. It is a fungus of the family Mucoraceae. It produces fluffy, white mycelia which is flat, mealy, chalky and pulverulent growth. It remains white even after the sporulation. Germination proceeded in two separable phases: Phase I (Swelling) and phase II (Germ tube protrusion). The optimal conditions for germination were 28°C and pH 5.0. It was observed that the

Table 1. Survey of Pesticides used in different places

Sample	Siripuram		Prathipadu		Chilakaluripet		Amaravathi	
	Pesticide Used	Trade Name	Pesticide Used	Trade Name	Pesticide Used	Trade Name	Pesticide Used	Trade Name
1	Acephate (organophosphate) Carbamate Pyrethroid group Monocrotophos (organophosphate)	Orthene(R) Avaunt Cypermethrin-25 E C Nuvacron	Acetamiprid Imidachloprid Quinolphos Chlorpyrifos Carbomate Spinosyn	Pride Confidor Ekalux Coroban Avaunt Tracer	Acetamiprid Imidachloprid Quinolphos Chlorpyrifos Carbomate Spinosyn	Pride Confidor Ekalux Coroban Avaunt Tracer	Alphamethrin (pyrethroid) Imidachloprid Quinolphos Acetamiprid Endosulphon	AXIS Confidor Ekalux Pride -
2	Spinosyns Acephate Pyrethroid group Monocrotophos (organophosphate)	Tracer Naturalyte (R) Orthene (R) Cypermethrin Nuvacron	Acetamiprid Cypermethrin Alphamethrin Spinosyn Carbomate Monocrotophos	Pride Cyrux Axis Tracer Avaunt Nuvacron	Acetamiprid Cypermethrin Alphamethrin Spinosyn Carbomate Monocrotophos	Pride Cyrux Axis Tracer Avaunt Nuvacron	Quinolphos Carbomate Acetamiprid Cypermethrin Spinosyn	Ekalux Avaunt Pride Cyrux Tracer
3	Monocrotophos Spinosyns Carbomate Acephate Chlorpyrifos Endosulphon	Nuvacron Tracer Naturalyte(R) Avaunt Orthene(R) Coroban	Monocrotophos Acetamiprid Chlorpyrifos Endosulphon Acephate -	Nuvacron Pride Coroban ——— Orthene(R)	Monocrotophos Acetamiprid Chlorpyrifos Endosulphon Acephate	Nuvacron Pride Coroban ——— Orthene(R)	Alphamethrin Cypermethrin Carbomate Spinosyn	AXIS CyruxAvaunt Tracer
4	Monocrotophos Carbomate Quinolphos Pyrethroid group	Nuvacron Avaunt Ekalux Cypermethrin	Acetamiprid Imidachloprid Quinolphos Monocrotophos Chlorpyrifos	Pride Confidor Ekalux Nuvacron Coroban	Acetamiprid Imidachloprid Quinolphos Monocrotophos Chlorpyrifos	Pride Confidor Ekalux Nuvacron Coroban	Chlorpyrifos Monocrotophos Acephate Quinolphos	Coroban Nuvacron Orthene (R) Ekalux
5	Monocrotophos Spinosyns Acephate Pyrethroid group Quinolphos	Nuvacron Tracer Naturalyte(R) Orthene(R) Cypermethrin Ekalux	Monocrotophos Acetamiprid Quinolphos Chlorpyrifos Carbomate	Nuvacron Pride Ecalux Coroban Avaunt	Monocrotophos Acetamiprid Quinolphos Chlorpyrifos Carbomate	Nuvacron Pride Ecalux Coroban Avaunt	Imidachloprid Monocrotophos Acephate Acetamiprid Spinosyn Endosulphon	Confidor Nuvacron Orthene (R) Pride Tracer-
6	Spinosyns	Tracer	-	Nuvacron	-	Nuvacron	Cypermethrin	Cyrux

	Acephate	Naturalyte(R)	Chlorpyrifos	Coroban	Chlorpyrifos	Coroban	Carbomate	Avaunt
	Pyrethroid group	Orthene(R)	Quinolphos	Ecalux	Quinolphos	Ecalux	Imidachloprid	Confidor
	Nicotinoids	Cypermethrin	Spinosyn	Tracer	Spinosyn	Tracer	Chlorpyrifos	Coroban
	(Acetamiprid)	Pride	Carbomate	Avaunt	Carbomate	Avaunt	Endosulphon	———
			Imidachloprid	Confidor	Imidachloprid	Confidor	Quinolphos	Ekalux
7	Spinosyns	Tracer	Acetamiprid	Pride	Acetamiprid	Pride	Acephate	Orthene
	Acephate	Orthene(R)	Chlorpyrifos	Coroban	Chlorpyrifos	Coroban	Quinolphos	(R)
	Chlorpyrifos	Coroban	Carbomate	Avaunt	Carbomate	Avaunt	Chlorpyrifos	Ekalux
	Carbomate	Avaunt	Acephate	Orthene	Acephate	Orthene	Alphamethrin	Coroban
			Quinolphos	(R)Ecalux	Quinolphos	(R)Ecalux	Acetamiprid	AXISPride
8	Acephate	Orthene(R)	Acephate	Orthene	Acephate	Orthene	Monocrotophos	Nuvacron
	Pyrethroid group	Cypermethrin	Chlorpyrifos	Coroban	Chlorpyrifos	Coroban	Cypermethrin	Cyrux
	Quinolphos	Ekalux	Monocrotophos	Nuvacron	Monocrotophos	Nuvacron	Quinolphos	Ekalux
			Spinosyn	Tracer	Spinosyn	Tracer	Imidachloprid	Confidor
			Carbomate	Avaunt	Carbomate	Avaunt		
9	Acetamiprid	Pride	Acetamiprid	Pride	Acetamiprid	Pride	Monocrotophos	Nuvacron
	Imidachloprid	Confidor	Spinosyn	Tracer	Spinosyn	Tracer	Spinosyn	Tracer
	Pyrethroid group	Cypermethrin	Acephate	Orthene	Acephate	Orthene	Quinolphos	Ekalux
	Monocrotophos	Nuvacron	Imidachloprid	(R)	Imidachloprid	(R)	Acetamiprid	Pride
			Carbomate	Confidor	Carbomate	Confidor	Cypermethrin	Cyrux
				Avaunt		Avaunt		
10	Carbomate	Avaunt	Cypermethrin	Cyrux	Cypermethrin	Cyrux	Acephate	Orthene
	Spinosyns	Tracer	Monocrotophos	Nuvacron	Monocrotophos	Nuvacron	Carbomate	(R)
	Endosulphon	Naturalyte	Chlorpyrifos	Coroban	Chlorpyrifos	Coroban	Chlorpyrifos	Avaunt
	Quinolphos	———	Acephate	Orthene	Acephate	Orthene	Spinosyn	Coroban
		Ekalux	Imidachloprid	(R)	Imidachloprid	(R)	Alphamethrin	Tracer
				Confidor		Confidor		AXIS

Table 2. Microorganisms collected in various soil samples of Guntur district

Sample	Siripuram	Prathipadu	Chilakaluripet	Amaravathi
1	*Aspergillus niger*, Saccharomyces cerevisiae, Fusarium oxysporum	*Bacillus subtilis*, Aspergillus flavus, Saccharomyces cerevisiae	*Bacillus subtilis*, Aspergillus niger, Trichoderma viride	*E.coli*, *Penicillium* notatum, Bacillus subtilis
2	*E.coli*, *Pseudomonas putida*, *Penicillium chrysogenum*	*Aspergillus terres*, *Pseudomonas aeruginosa*, *Mucor racemosus*, racemosus, Bacillus subtilis	*Bacillus subtilis*, *Saccharomyces cerevisiae*, Rhizopus microsporus	*Bacillus subtilis*, *Aspergillus niger*, Fusarium solani Mucor indicus
3	*Bacillus cereus*, Fusarium solani, Aspergillus flavus	*Penicillium* chrysogenum, Fusarium solani, Bacillus subtilis, Trichoderma viride	*Penicillium* chrysogenum, Fusarium oxysporum, Mucor racemosus.	*Pseudomonas* aeruginosa, E.coli, Fusarium oxysporum
4	*Mucor racemosus*, Trichoderma viride, E.Coli	*Rhizopus* microsporus, E.Coli, Bacillus subtilis, Fusarium oxysporum, Pseudomonas putida	*E.Coli*, *Pseudomonas* putida, Fusarium solani, Rhizopus microsporus Saccharomyces cerevisiae	*Saccharomyces* cerevisiae, Mucor indicus , Trichoderm a viride
5	*E.coli*, *Pseudomonas* aeruginosa, Rhizopus microsporus, Aspergillus niger	*Bacillus cereus*, Pseudomonas aeruginosa, Rhizopus microsporus, Fusarium oxysporum	*Pseudomonas putida*, Bacillus subtilis, Fusarium oxysporum, Aspergillus niger	*Bacillus subtilis*, Mucor indicus *Aspergillus flavus* E.Coli
6	*Achromobacter*, Bacillus subtilis, Penicillium notatum, Saccharomyces cerevisiae	*Bacillus subtilis*, E.Coli, Aspergillus niger, Pseudomonas aeruginosa	*Bacillus subtilis*, E.Coli Rhizopus microsporus, Penicillium chrysogenum	*E.coli*, Rhizopus microsporus,, Saccharomyces cerevisiae
7	*Mioxococcus*, Alternaria solani, Aspergillus niger, Trichoderma viride	*Mucor racemosus*, Aspergillus flavus, Penicillium chrysogenum putida,Penicillium	*Bacillus subtilis*, Micrococcus, Fusarium solani,	*E.coli*, Pseudomonas Mucor racemosus notatum, Trichoderma viride
8	*Bacillus subtilis*, Pseudomonas putida, Rhizopus stolanifer, Trichoderma viride	*E.Coli*, Rhizopus microsporus, Fusarium solani	*Pseudomonas putida*, Bacillus subtilis, Mucor racemosus, Aspergillus terres	*Bacillus subtilis* , E.coli, Saccharomyces cerevisiae, Fusarium solani
9	*E.coli*, Aspergillus flavus, Fusarium solani	*Bacillus subtilis*, Pseudomonas aeruginosa, Aspergillus flavus	*Achromobacter*, Penicillium notatum, Rhizopus microsporus, Saccharomyces cerevisiae	*Pseudomonas* putida, Alternaria solani Fusarium oxysporum
10	*Pseudomonas* aeruginosa, Aspergillus terres	*Bacillus subtilis*, Myxococcus, Penicillium notatum	*Pseudomonas putida*, Aspergillus niger, E.Coli	*Bacillus cereus* E.coli, Aspergillus flavus Mucor indicus

sporangiospores contain insufficient endogenous carbon for swelling or germination to occur in distilled water. Initial swelling during phase-I occurred only could be due to the presence of a suitable carbohydrate. Subsequent production of germ tubes during phase II required exogenous sources of both carbon and nitrogen. The observations noticed were in accordance with the findings of Richard and Dale (1984) and Kobayasi *et al.* (1992). Basing on the microscopic observation and culture characteristics, it can be concluded that the screened and identified species were confirmed to be *Rhizopus oligosporus.*

Table 3. Growth of Test fungi on different media

S. No	Medium	*R. oligosporus* (Spore count per ml)
1	Potato Dextrose broth	4.74×10^6
2	Czapekdox broth	2.84×10^6
3	Sabourauds broth	0.64×10^6

Table 4. Effect of Physico-chemical parameters (Temperature, P^H and Salt concentration) on the growth of test fungi

Physico-chemical parameter	Varied value	*Rhizopus oligosporus*
Temperature(^oC)	25	++
	28	++
	30	++
	35	+
	37	+
	40	-
	45	-
	55	-
pH	2.5	-
	3.5	-
	4.5	+
	5.5	++
	6.5	+
	7.0	+
	7.5	+
	8.5	+
	9.5	-
Salt concentration (%)	0	+
	0.5	+
	2.5	+
	5	-
	7.5	-
	10	-

Table 5. Optimization of media in presence of Pesticide (Malathion) by *Rhizopus oligosporus*

Medium	% of degradation
Potato Dextrose Broth	53.16
Czapekdox Broth	44.91

Microscopic structural and growth characterization, scanning electron microscopic studies and molecular characterization basing on DNA coding for 18s rRNA sequences of the test organisms capable to degrade pesticides were performed and it was concluded that the test fungus is *Rhizopus oligosporus*. It is a fungus of the family Mucoraceae. It produces fluffy, white mycelia which is flat, mealy, chalky and pulverulent growth. It remains white even after the sporulation. Germination proceeded in two separable phases: Phase I (Swelling) and phase II (Germ tube protrusion). The optimal conditions for germination were 28°C and pH 5.0. It was observed that the sporangiospores contain insufficient endogenous carbon for swelling or germination to occur in distilled water. Initial swelling during phase-I occurred only could be due to the presence of a suitable carbohydrate. Subsequent production of germ tubes during phase II required exogenous sources of both carbon and nitrogen. The observations noticed were in accordance with the findings of Richard and Dale (1984) and Kobayasi *et al.* (1992). Basing on the microscopic observation and culture characteristics, it can be concluded that the screened and identified species were confirmed to be *Rhizopus oligosporus.*

The scanning electron microscopic studies confirmed that the screened species were confirmed to be as *Rhizopus oligosporus*. As the microscopic structural identification and the growth characterization cannot be attributed to confirmed degradative capacity of the fungal organisms, it requires further characterization. Hence, the fungal confirmation study was extended using molecular techniques based on the DNA coding for 18s rRNA sequences.

The genomic DNA of the isolated fungal organisms was subjected for the isolation of DNA coding for 18s rRNA by using PCR (Fig.3). The

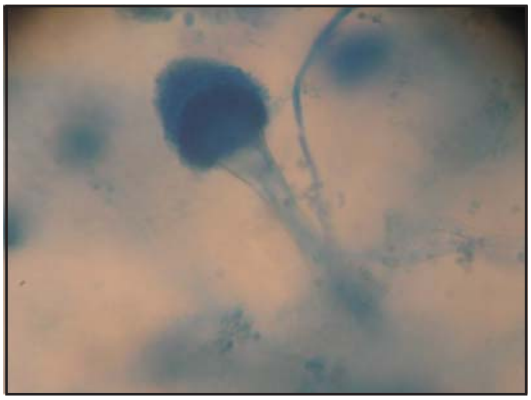


Fig. 1. Micrograph of *Rhizopus oligosporus*

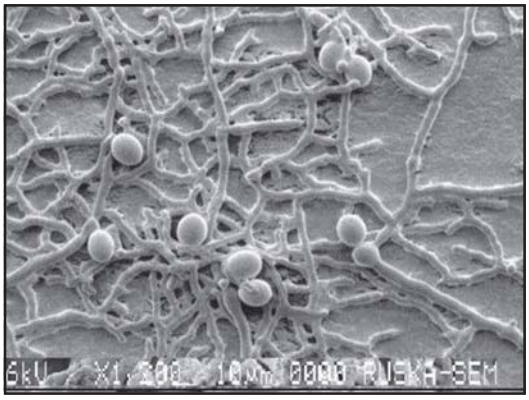
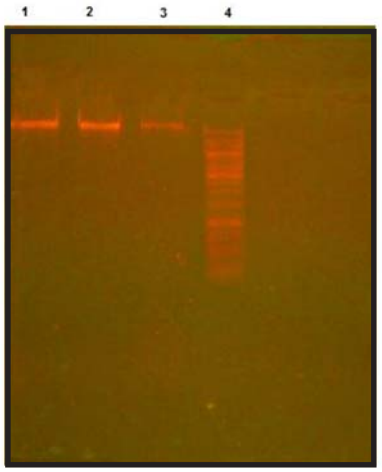


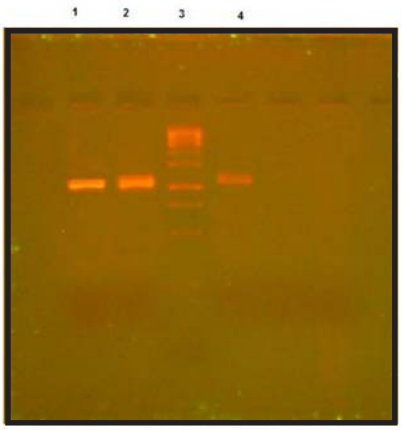
Fig. 2. Scanning Electron Micrograph of *R.oligosporus*



Well 2: Genomic DNA of *Rhizopus oligosporus*

Well 4: DNA Ladder

Fig. 3. Agarose gel showing Genomic DNA



Well 3: DNA Ladder

Well 4: Amplified DNA of *Rhizopus oligosporus*

Fig. 4. Agarose gel showing the amplified 18sDNA

bands were cut and eluted and the DNA so obtained was subjected for sequencing.

The sequencing analysis of isolated DNA has revealed that the amplified DNA corresponds to 600 base pairs for *Rhizopus oligosporus*. The DNA sequence obtained for the different isolated fungi is shown in Fig. 5.

The sequences obtained in the present investigation were subjected to BLAST hit with the sequences of NCBI database. Basing on the alignment occurred, aligned sequences at the maximum degree were selected and the phylogenetic tree was constructed. The phylogenetic tree so obtained was given in Fig. 6.

The microscopic structural and growth characterization studies together with scanning electron microscopic studies and amply supported by the molecular characterization basing on the sequence analysis of DNA coding for 18s rRNA of the test fungal organisms, it was confirmed that the isolated fungi belongs to *R. oligosporus* (Fig. 5) and further confirmed that these fungi are capable to degrade the organophosphorus pesticides. The study was extended to develop an effective biodegradation model.

Growing fungi on laboratory media is a costly and tedious process. From the observations, it was evident that the Potato Dextrose broth has produced more number of spores followed by Czapekdox broth and least number of spores was produced in Sabourauds broth

The OP pesticides are known to cause adverse effects to the ecosystem and mankind. They affect the nervous system by inhibiting a critical enzyme called acetylcholinesterase, involved in nerve signaling. An appropriate balance of acetylcholinesterase is needed to help with muscle control, including muscles of the respiratory tract, heart, and arms and legs. The OP pesticides can cause damage to the reproductive system (sperm defects in men, and decreased pregnancies and survival of offspring in animals) chest pain, respiratory distress, incoordination, vomiting, damage to the central and peripheral nervous systems and death due to respiratory distress or cardiac arrest at high levels of exposure. Keeping the significance of effective biodegradation of OP pesticides by fungi, in the present study an attempt was made to optimize the various physico-chemical parameters for efficient growth of cultured fungal organisms and inturn to enhance the biodegradation.

After inoculation of *Rhizopus oligosporus* in the Potato Dextrose broth, incubated at different temperatures (25^{0}C, 28^{0}C, 30^{0}C, 35^{0}C, 37^{0}C, 40^0 C, 45^{0}C and 55^{0}C) for 4 days, maximum growth was observed at temperatures of 25^{0}C, 28^{0}C and 30^{0}C whereas minimal growth was observed at temperatures 35^{0}C and 37^{0}C and there was no growth above temperatures of 40^{0}C. This reveals that the optimum temperature for *Rhizopus oligosporus* was 25^{0}C - 30^{0}C (Table 4).

GGAAGGATCATTAACTAATGTATTGGCACTTTACTGGGATTTACTTCTCAGTATTGTT
TGCTTCTATACTGTGAACCTCTGGCGATGAAGGTCGTAACTGACCTTCGGGAGAGAC
TCAGGACATATAGGCTATAATGGGTAGCTGTTCTGGGGTTTGATCGATGCCAATCAG
GATTACCTTTCTTCCTTTGGGAAGGAAGGTGCCTGGTACCCTTTACCATATACCATGA
ATTCAGAATTGATATAATAACAACTTTTAACAATGGATCTCTTGGTTCTCGCATCGAT
GAAGAACGTAGCAAAGTGCGATAACTAGTGTGAATTGCATATTCGTGAATCATCGA
GTCTTTGAACGCAGCTTGCACTCTATGGATCTTCTATAGAGTACGCTTGCTTCAGTAT
CATAACCAACCCACACATAAAATTTATGTGGTGATGGACAAGCTCGGTTAAATTTAA
TTATTATACCGATTGTCTAAAATACAGCCTCTTTGTAATTTTCATTAAATTACGAACT
ACCTAGCCATCGTGCTTTTTTGGTCCAACCAAAAAACATAATCTAGGGGTTCTGCTA
GCCAGCAGATATTTTAATGATCTTTAACTATGATCTGAAGTCAAGTGG

Fig. 5. 18S ribosomal RNA gene of *Rhizopus oligosporus*

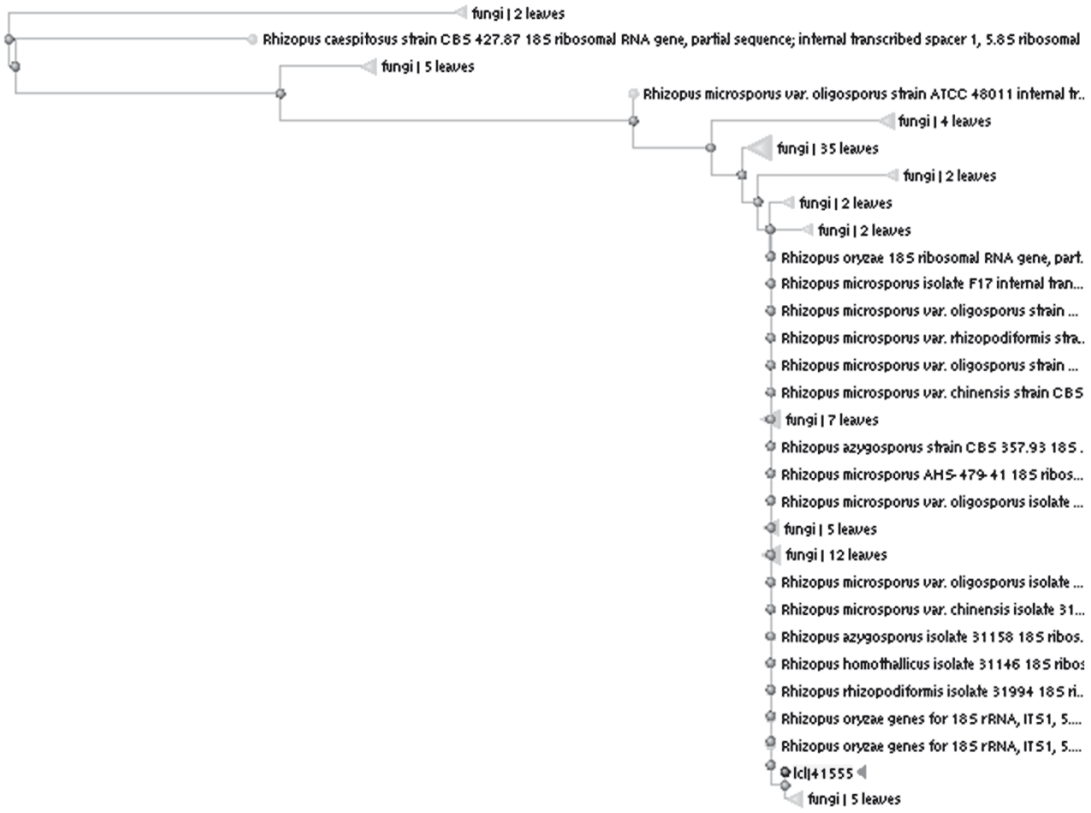
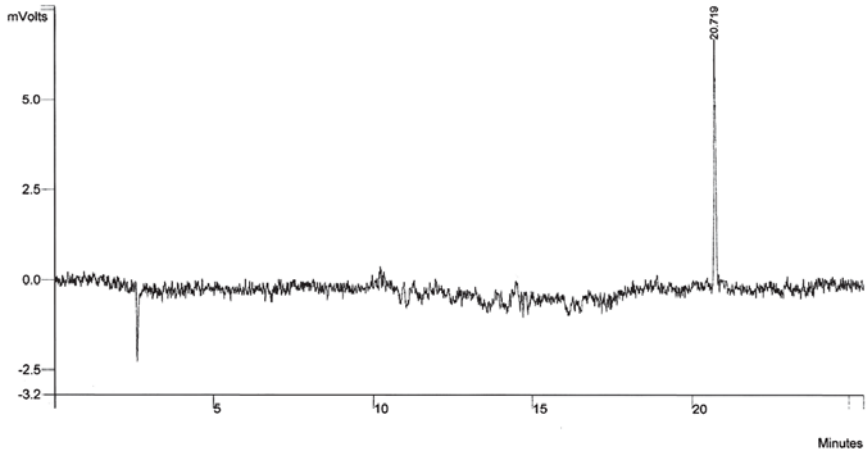


Fig. 6. Phylogenetic tree of *Rhizopus oligosporus*



Peak No	Ret. Time (min)	Area (counts)	Peak Name
1	20.719	28815	
		28815	Totals

Fig. 7. Gas chromatograph of Malathion standard

Potato Dextrose broth with different pH (2.5, 3.5, 4.5, 5.5, 6.5, 7, 7.5, 8.5, 9.5) was inoculated with *Rhizopus oligosporus* and incubated for 7 days. Growth was not observed in the flasks with pH 2.5, and 3.5. Maximum growth was observed at pH 5.5 and moderate growth was observed at pH 4.5, 6.5, 7.0, 7.5 and 8.5 and no growth was observed at pH 9.5. These results indicate that the optimum pH for the growth of the selected fungus at these conditions was 5.5 and moderate growth in between 6.5 to 8.5 (Table 4).

Growth medium with different salt concentrations (0%, 0.5%, 5%, 7.5% and 10%) were inoculated with *Rhizopus oligosporus* and incubated for 7 days. The growth was observed in all the inoculants of NaCl concentration of 0%, 0.5% and 2.5% where as the growth was not observed in 5%, 7.5% and 10% of NaCl concentration. This gives the information that the growth possibility for our desired fungi was upto 2.5% Sodium chloride (NaCl) concentration only (Table 4).

The degradation capability of selected fungal species, *Rhizopus oligosporus* on selected organophosphate pesticide, Malathion was confirmed through Gas chromatography. The biodegradation of Malathion with the above selected fungal species for medium composition for each combination of a particular species and pesticide was investigated.

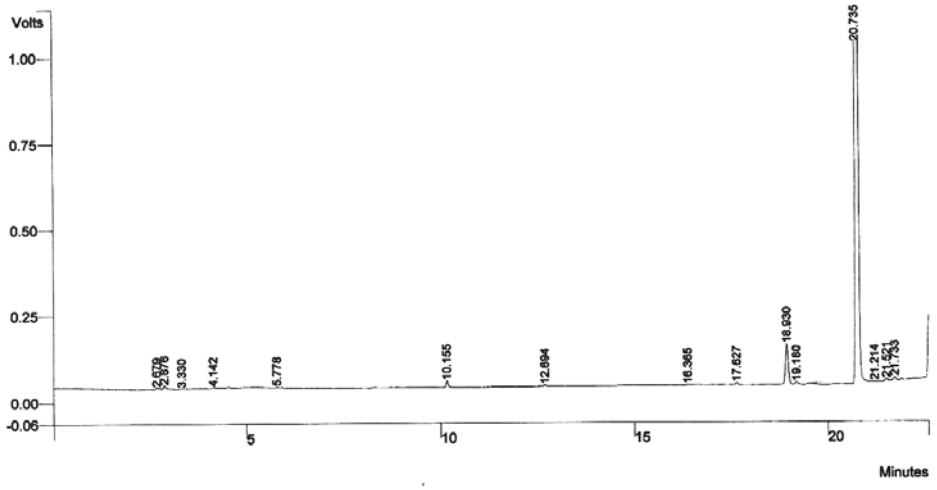


Fig. 8. Gas chromatograph of Malathion control

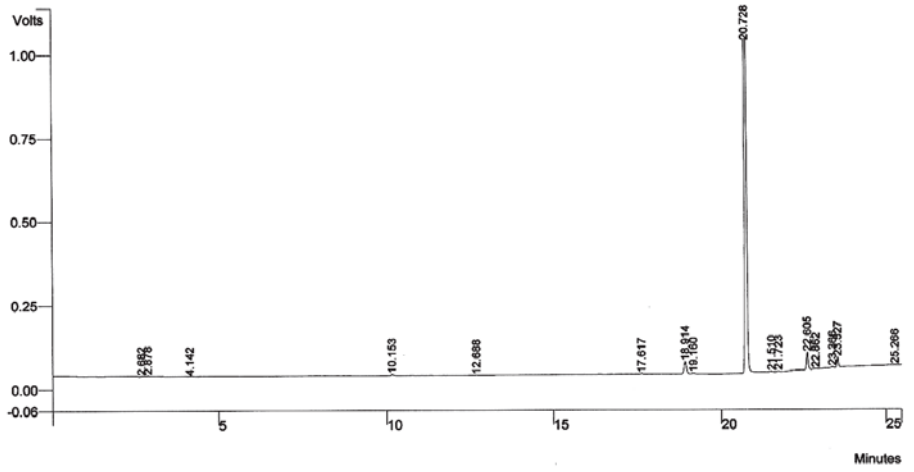


Fig. 9. Gas chromatograph of Malathion sample

Malathion was degraded to the maximum extent by *Rhizopus oligosporus*. In both the media, i.e., Potato Dextrose broth and Czapekdox medium, similar pattern of degradation of the respective organophosphate pesticides was noticed. It was also evident that the maximum percentage of degradation was seen in Potato Dextrose broth by the test fungi on the used organophosphate pesticides (Table 5).

The pesticide residues were extracted from the culture medium by liquid-liquid extraction method and the analysis of the pesticide residue samples was carried out by using Gas chromatography technique.

It was clearly evident that the cotton cultivated soils are prone for heavy pesticide residues due to the continuous usage and it is quite possible that these pesticides can enter into food chain and can cause several deleterious effects even to human beings. Results of the present study may fit the findings on the ability of fungi to degrade pesticides and introduce some new information on mineralization of organic constituent of pesticides. This will decrease the side effects of pesticides on the non-target microorganisms that play an important role in soil fertility. In the light of this, an attempt has to be made further, to investigate the possibility of biodegradation of various organophosphorus pesticides using the fungal isolate *R. oligosporus*.

CONCLUSION

The study elucidated that the selected fungal species can be employed as effective biodegrading species. By further optimizing the different culture conditions, the selected fungal species, *R. oligosporus* can be used as an effective biodegrading agent in cotton fields. Also it was found to be an efficient alternative for the control of environmental pollution as it is screened from the soils with 7-8 years of cotton growing history and was isolated in presence of selected pesticide.

REFERENCES

1. Alexander M., Biodegradation and Bioremediation, Academic Press, San Diego, 1994; pp 139.
2. Aneja, K.R., *Experiments in Microbiology, Plant Pathology, Tissue Culture and Mushroom Production Technology*; New Age International Publishers; Third Edition. 2003; 221-240.
3. Black, C.A., Evans, D.D., Ensminger, L.E., Methods of soil analysis. American Society of Agronomy. Incorp. Publishers, Madison, Wisconsin. 1965; pp. 1149-1178.
4. Hayes, W.J., *Pesticides Studied in Man*. Baltimore, MD: Williams and Wilkins, Baltimore, 1982; pp.49.
5. Jackson, M.L., Soil chemical analysis. Prentice Hall, Englewood Cliffs. 1958; pp 227-255.
6. Kobayasi S., Okazaki N. and Koseki T. Purification and characterization of an antibiotic substance produced from *Rhizopus microsporus*, IFO 8631, 1992.
7. Kurzel, R.B., Certrulo, C.L., The effect of environmental pollutants on human reproduction, including birth defects. *Environ. Sci. Technol.* 1981; **15**: 626–631.
8. Mullis, K.B., The Unusual origin of Polymerase Chain reaction. *Scientific American*. 1990; 36-43.
9. Pimentel, D., Effects of pesticides on the environment. 10th *Intl. Congress on Plant Protection*. Crydon, U.K. 1983; **2**: 685-691.
10. Ramesh Babu, T., Srinivasa Rao, Ch., Narasimha Reddy, K., Temperature programmed gas chromatography method for the determination of pesticide residues. *Indian Journal of Plant Protection*. 2001; **29**(1 & 2): 161-164.
11. Richard D. M. and Dale W. G., Germination of *Rhizopus microsporus* Sporangiospores, *Appl Environ Microbiol*, 1984; **48**(6): 1067–1071.
12. Singh, V.K., Singh, A., Singh, S.P., Effect of interaction of herbicides on chlorophyll and mineral content of rice leaves. *J. Agric. Res.* 1987; **2**: 175–177.